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# A review on car-t cell therapy- traditional strategies for cancer treatment

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## Abstract

Cancer is the world's top cause of death. Numerous cytotoxic immunotherapies and traditional treatments have been created and introduced to the market. The development of a viable immunotherapy that targets cancers at both the cellular and genetic levels is necessary due to the complicated behaviour of tumors and the involvement of multiple genetic and cellular variables in tumorigenesis and metastasis. A new therapeutic T cell engineering technique called chimeric antigen receptor (CAR) T cell treatment involves in vitro altering patient blood-derived T cells to express synthetic receptors that are directed against a particular tumor antigen. The major histocompatibility complex is not involved in these; instead, the tumor antigen is directly identified. The use of this therapy in the last few years has been successful, with a reduction in remission rates of up to 80% for hematologic cancer, particularly for acute lymphoblastic leukemia (ALL) and non-Hodgkin lymphomas, such as large B cell lymphoma. CAR therapy has the potential to offer a rapid and safer treatment regime to treat non-solid and solid tumors. CAR-T cell therapy's most significant benefit over other cancer treatments is its quick time intervention a single infusion of CAR T cells. Additionally, the patient only has to be properly cared for and observed for two to three weeks. CAR T cell therapy is considered a "drug of the present day," and because the cells may live in the host body for a long time and have the capacity to continuously identify and eliminate cancer cells after relapse, its effectiveness may last for decades. The present review insight into the structure and evolution of chimeric antigen receptors, we then report on the tools used for production of CAR-T cells. Finally, we address the challenges posed by CAR-T cells.

**Keywords:** Cancer; CAR-T cell; Chimeric antigen receptor; Acute lymphoblastic leukemia

## 1. Introduction

Chimeric antigen receptor (CAR T) cell therapy is a revolutionary new pillar in cancer treatment (1). Although treatment with CAR T cells produced remarkable clinical responsible with certain subsets of B cell leukemia or lymphoma many challenges limit the therapeutic efficacy of CAR-T cell in solid tumour and haematological malignancy (2). CARs are engineered synthetic receptor that functions to redirect lymphocytes, most commonly T cells, to recognize and eliminate cells expressing a specific target antigen. When we pioneered the first CAR design in the late 1980s and early1990s, it was widely known that T cell Are very powerful effectors in the fight against cancer, but the application of these cells to cancer patients suffered from two major limitation(3). First T cell recognition depends on the expressions of major histocompatibility complex molecules and antigen processing machinery, and many tumours silence these pathways as part of their escape from immune recognition(4). second many tumours that do not express costimulatory molecules required for triggering the full potency of T cell often render tumour-specific T cell nonfunctional researchers have designed CARs to offer an alternative to conventional T cell receptors (TCRs) and circumvent these hurdles(1,3). The unprecedented success of anti CD19 CAR-T cell therapy against B cell malignancies resulted in its approved by the US food and drug administration (FDA) in 2017.however, there are major limitations to CAR-T cell therapy that still must be addressed including life-threatening CAR-T associated toxicities, limited efficacy against persistence, poor trafficking and tumour infiltration, and the immunosuppressive ((2,5).

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### 1.1. Background

Chimeric antigen receptors (CARs) are engineered receptors that provide immune effector cells (T cells) with a customized specificity (6). CARs consist of three components: an extracellular domain for antigen recognition derived from a single-chain variable fragment (scFv) of an antibody, a transmembrane segment, and an intracellular T cell activation domain known as CD3 (7). The purpose of CAR-T cell therapy is to guide a patient's or donor's T cells to precisely locate and eliminate cancer cells. This approach holds significant potential for treating hematologic cancers as well as solid tumors, without being restricted by major histocompatibility complex (8).

Immunotherapy has revolutionized cancer treatment, offering a flash of hope to patients facing late-stage metastatic tumors. Science magazine acknowledged its impact, designating it as the "Breakthrough of the Year" in 2013(9).

### 1.2. Engineering of CAR-T cells

Engineered receptors named chimeric antigen receptors (CARs) have the ability to transfer an arbitrary specificity onto an immune effector cell (T cell). CARs are composed of three components: a transmembrane domain, an intracellular T cell activation domain of CD3, and an extracellular antigen recognition domain of the single-chain fragment variant (astx), which is generated from an antibody(10).

The goal of CAR-T cell therapy is to direct T cells from a donor or patient to specifically target and kill tumer cells. Solid tumers and haematologic malignancies without significant constraints on the major histocompatibility complex can benefit greatly from this treatment (11).

### 1.3. Structure of CAR-T cells:

CARs involves mainly ectodomain, transmembrane domain and endodomain(12).

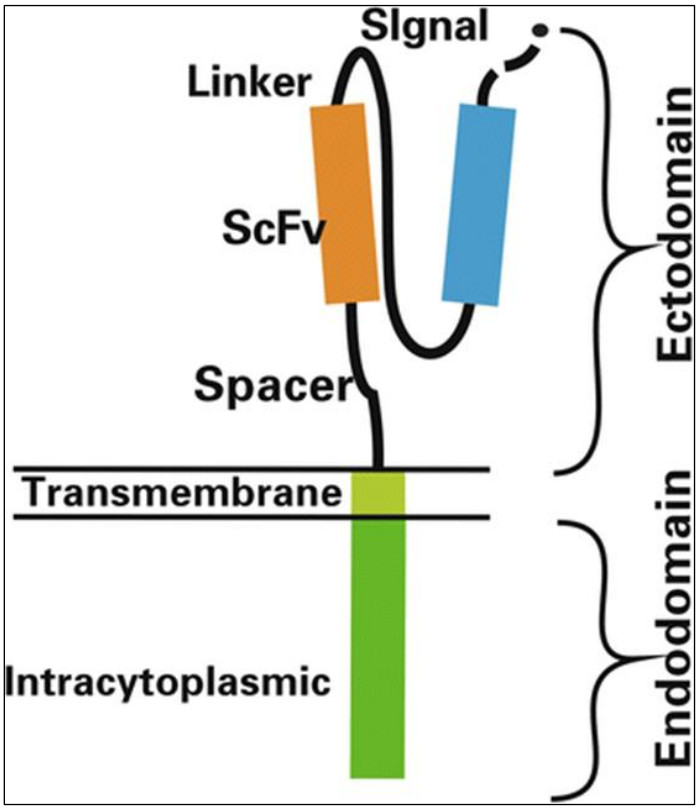


**Figure 1** Structure of CAR-T cell

### 1.4. Ectodomain

The segment of a membrane protein that is exposed to the outside environment and not located within the cytoplasm is referred to as the ectodomain. In this instance, the signal peptide, the antigen recognition area, and the spacer constitute the ectodomain (7, 8).

A signal peptide's role is to direct the developing protein into the endoplasmic reticulum (13). The variable regions of the heavy and light chains of an antibody are connected through a flexible linker to create the single-chain variable fragment (scFv), which acts as the signal peptide for the ectodomain in a CAR. Any singular antigen capable of binding to targets with high affinity can be detected by an antigen recognition domain, frequently found as a single-chain variable fragment (scFv) with a fundamental ectodomain and additional specialized recognition components (14, 15). The spacer acts as a bridge between the transmembrane domain and the antigen binding domain. The hinge region of IgG1 represents the simplest form of spacer and is sufficient for most scFv-based constructs.

### 1.5. Transmembrane domain

The transmembrane domain, consisting of a hydrophobic alpha helix that extends across the membrane, represents the closest portion of the endodomain to the membrane (6, 8, 9). There is a relationship between the stability of the receptor and the transmembrane domain. The artificial TCR could integrate with the native TCR when the native CD3-zeta transmembrane domain is available (16, 17). At present, the most stable receptor is the transmembrane domain of CD28.

### 1.6. Endodomain

The most commonly found component of the endodomain, which is the operational end of the receptor, is CD3ζ, which includes three immunoreceptor tyrosine-based activation motifs (ITAMs)(8,9). A signal is transmitted to the T cell when the receptors cluster and activate upon recognizing an antigen. Co-stimulatory signaling is essential during this phase (17).

### 1.7. Evolution of CAR-T cells

Based on the endodomain's structure, CAR-T cells can be categorized into four generations since the first creation of CARs in 1989. A great illustration of how fundamental research can be applied in the clinic is the development of CAR treatment.

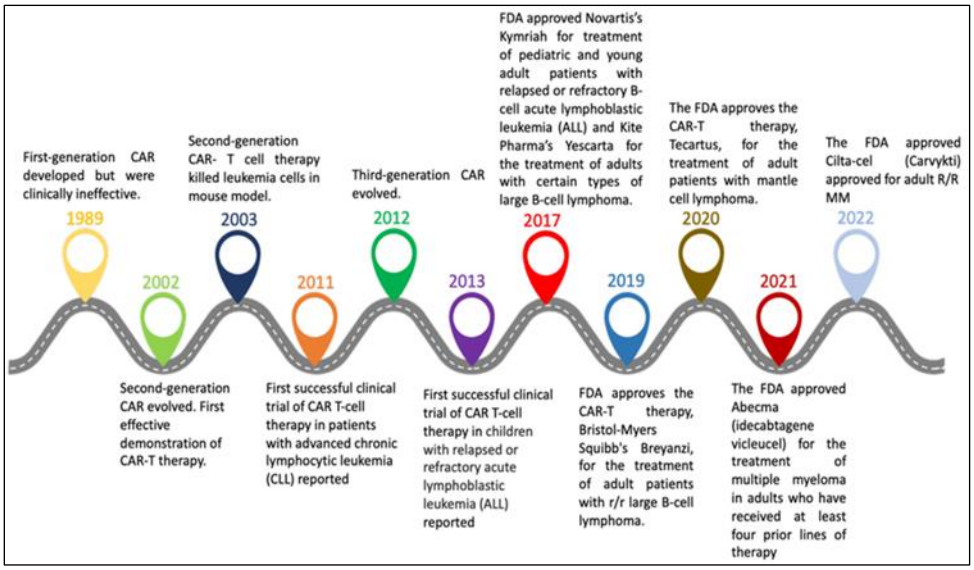


**Figure 2** Evolution of CAR-T cell

### 1.8. First generation

In the first generation of CARs, signals from the endogenous T cell receptor (TCR) were primarily transmitted by a single structure derived from the CD3 ζ-chain or FcεRIγ from the intracellular domain (17, 18). However, to effectively eliminate tumor cells, exogenous IL-2 needed to be administered because these CAR-T cells couldn't produce sufficient interleukin-2 (IL-2) on their own. Therefore, the combined use of cytokines proved advantageous for the initial generation of CAR-T cell therapies that utilized single-chain receptors (19).

Recent studies indicate that the apoptotic signal may be mitigated by removing phosphorylation from ITAM A and C in the CD3ζ signaling portion, which benefits the ongoing expression of the transgene (20, 21). Nonetheless, more extensive clinical research has been carried out with CD3ζ-chained CAR-T cells in comparison to FcεRIγ-chained CAR-T

cells. This might be attributed to the presence of one ITAM in the FcεRIγ chain versus three in the CD3ζ chain. Conversely, although CAR-T cells utilizing the CD3ζ chain demonstrated lower expression levels in vitro, they proved more effective at activating T cells and eliminating tumor cells. The transmembrane domain of CAR-T cells comprises a homologous or heterologous dimer of CD3, CD8, and CD28. This receptor is capable of facilitating optimal cellular activation through CAR dimerization and its functional link with the endogenous TCR. Various cancers have been treated with CD10-CAR-T cells, scFv(G250)-CAR-T cells, GD2-CAR-T cells, alpha-folate receptor (FR)-CAR-T cells, and carcinoembryonic Ag-specific CD3ζ (MFEζ)-CAR-T cells(22–27). However, due to constrained proliferation, a limited lifespan in vivo, and inadequate cytokine release, most early experiments involving first-generation CAR-T cells did not produce the expected outcomes.

### 1.9. Second generation

T cell activation is commonly described as a process requiring two signals. This process involves three primary types of receptors: co-stimulatory receptors, cytokine receptors, and T-cell antigen receptors. The initial signal is generated when the T-cell receptor (TCR) recognizes the antigenic peptide-MHC complex present on antigen-presenting cells. The secondary signal comes from a co-stimulatory molecule, such as CD28/B7, which promotes the production of IL-2, crucial for completing T cell activation and preventing cell death. Naïve T cells, even when stimulated by an antigen, cannot perform their normal functions without the co-stimulatory signal. Therefore, CARs that consist solely of the CD3ζ sequence cannot effectively activate CAR-T cells in the absence of this co-stimulatory signal. To provide additional signals to T cells, second-generation CARs incorporate intracellular signaling domains from various co-stimulatory protein receptors, such as CD28 or 4-1BB and OX40, into the cytoplasmic tail of the CARs. These modifications can improve the proliferation, effectiveness, and durability of CAR-T cells, as well as prolong their lifespan *in vivo* (28–30).

In addition to being essential for the formation of memory and effector cells, CD28-mediated co-stimulation influences the proliferation and survival of lymphocytes. CD134 can boost IL-2 production and support proliferation. The ability of CD137 to maintain the signaling of T cell responses is vital for both the survival of T cells and the memory of CD8+ T cells (31–33). When used in the treatment of B cell cancers, the scFvCD19-CD137-CD3-CAR-T cells, MOv19-BBζ-CAR-T cells, and scFvCD19-CD28-CD3ζ-CAR-T cells demonstrated better outcomes compared to the first generation (34, 35). Although direct comparisons are lacking, it seems that 4-1BBζ-CAR-T cells have a longer persistence than CD28ζ-CAR-T cells (36). CD28ζ-CAR-T cells are capable of stimulating, developing, and expanding in a consistent manner (37). Conversely, early exhaustion associated with 4-1BBζ-CAR-T cells may limit their antitumor effectiveness (38, 39).

### 1.10. Third generation

To enhance potency through increased cytokine production and improved killing capabilities, various signaling domains, including CD3ζ-CD28-OX40 or CD3ζ-CD28-41BB, were combined to develop third-generation CARs (40). While scFv CD20-CD28-CD137-CD3ζ-CAR-T cells and HER2-CAR-T cells were used to treat lymphoma and colon cancer, the outcomes did not surpass those achieved with second-generation therapies (41, 42). It is possible that the limited number of cases studied contributed to this result. Therefore, further research is necessary to evaluate the effectiveness and safety of these treatments, and the selection of co-stimulatory molecules plays a vital role.

### 1.11. Fourth generation

T cell redirected for universal cytokine-mediated killing (TRUCKs) refers to the fourth-generation CARs, developed by integrating IL-12 into the foundational design of second-generation constructs. TRUCKs enhance T-cell activation and also recruit innate immune cells to target and eliminate cancer cells that do not express antigens within the treated area. Exploring how TRUCKs influence the tumor microenvironment through the controlled release of transgenic immune modifiers could provide valuable insights. Additionally, these TRUCK T cells show efficacy in managing autoimmune disorders, metabolic issues, and viral infections (43).

When taken as a whole, these subsequent versions of CAR-T cell therapy have greatly increased interest in cancer treatment (44).

### 1.12. Mechanism of action

Host t cells are collected and are genetically modified ex vivo to express a CAR targeting a tumour specific antigen. The car construct consists of an extra cellular domain with a single chain variable fragment that targets the antigen of interest as well as an intra cellular domain that construct in the T cell membrane an initiate an intracellular signalling cascade (45).

The patient's mononuclear cells are collected, which is typically accompanied by leukapheresis. Manufacturers of CAR T cells is a complex, expensive, and time-intensive process, The T-cell subset is enriched, generally modified to express the CAR of interest, sub sequent expanded ex vivo, and stored until use (46). Genetic modification of t cells is accomplished by one of three major methods currently used in clinical practice: -retroviral vectors, lentiviral vectors, or the transposon system after immunophenotypic confirmation of successful genetic modification, a growth platform is used to rapidly expand the CAR- T cells (47).

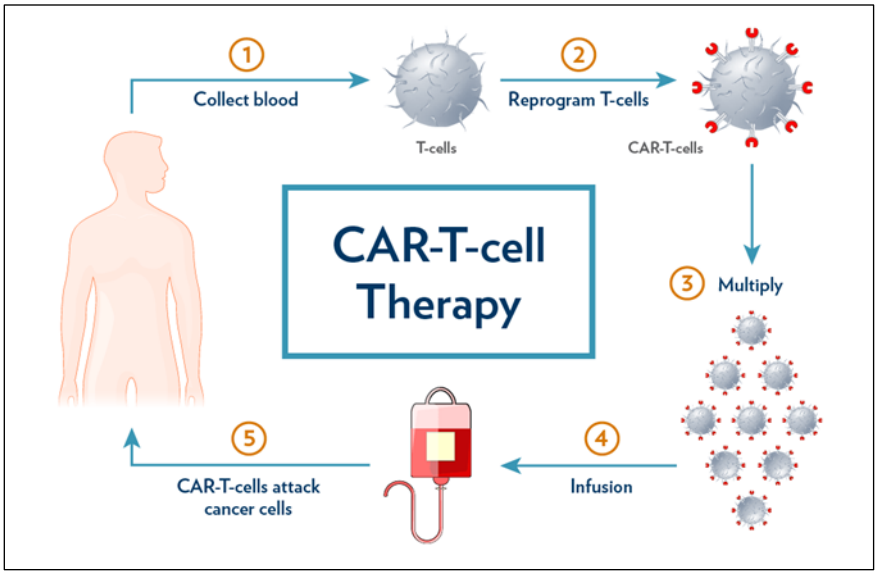


**Figure 3** Mechanism of CAR-T cell therapy

### 1.13. Tools of transduction for CAR-T cells

To introduce a foreign gene into human cells, a specific tool is necessary. Currently, gene integration using vectors can be carried out through two primary methods: viral systems and non-viral systems.

Viral vectors are preferred in gene therapy for both research and clinical applications due to their high efficiency in transferring genes, quick ability to achieve the necessary quantity of cultured T cells, and the variety of viruses available, each with distinct expression profiles. Most viral systems can incorporate genes from interesting and valuable cells and can provide the structural proteins and enzymes essential for the generation of infectious viral particles carried by vectors. Examples of viral vectors include retroviruses like lentiviruses, adenoviruses, and adeno-associated viruses. Among these, genetically modified retroviruses are the most commonly used for gene delivery (48). A group of retroviruses is known as Retroviridae. This group varies in aspects such as host range, pathogenic potential, structure of the genome, sequences of amino acids and nucleotides, and host range. Nevertheless, the viral vectors can pose health risks. The achieved titer is inadequate, the ability to carry is limited, and the insertion mutations used to stimulate the immune response may lead to cancer and toxicity (48, 49). The benefits of non-viral gene therapy compared to other cancer treatments include enhanced effectiveness, precise targeting, non-infectious nature, unlimited carrier capacity, customizable chemical composition, and high production availability. Examples of non-viral vectors comprise molecular conjugates, liposomes, polymerizers, and naked DNA(50,51). Minicircle DNA vectors represent a novel class of non-viral vectors created in bacteria from a parental plasmid, capable of producing high levels of transgene expression in vivo while lacking plasmid bacterial DNA sequences. This method is applicable in clinical settings (52, 53).

### 1.14. Production of CAR-T cell

The process of creating CAR-T cells involves multiple steps. Additionally, conducting quality control assessments at every stage of the protocol is essential (54).

Leukocytes are obtained from the patient or donor through a process called leukapheresis (55). Next, T cells are isolated and cleaned to distinguish them from the leukocytes. Then, specific antibody bead conjugates or markers are employed to separate the T cell subsets based on their CD4/CD8 composition. The final step involves culturing the T cells to activate them. This procedure includes purifying the autologous antigen-presenting cells (APCs) from the patient or donor, or utilizing beads that are coated with anti-CD3/anti-CD28 monoclonal antibodies, or just anti-CD3 antibodies,

with or without feeder cells and growth factors such as IL-2, which is commonly used because it enhances rapid T cell proliferation; further adjustments to the culture conditions are made to direct T cells towards a specific phenotype (56).

Viral vectors that are incorporated in CARs guide the reverse transcription of RNA into DNA, which then permanently integrates into the genome of the patient's cells. In the activation process, the viral vector is eliminated from the culture through media exchange and/or dilution. Due to their better profile for integration sites, lentiviral vectors are more commonly utilized in clinical trials compared to gammaretroviral vectors.(57)

mRNA transfection and the Sleeping Beauty transposon system represent additional methods. However, many questions remain unresolved, such as the requirement for multiple infusion cycles with temporary mRNA transfection and the risks of insertional mutagenesis and transposon remobilization when using the Sleeping Beauty transposon system (58).

CAR-T cells are cultivated using three distinct bioreactor culture systems: CliniMACS Prodigy, G-Rex, and WAVE Bioreactor (59). A significant drawback of the first two methods is that the flask must be opened during cell inoculation. Conversely, the CliniMACS Prodigy system serves as a comprehensive tool that effectively enriches, activates, transduces, and cultivates the cells (60). Once the cells reach the necessary quantity for therapeutic use, they are extracted and administered to the patients.

### 1.15. The clinical success of CAR-T cell therapy

A patient at NCI suffering from advanced follicular lymphoma and patients at MSKCC with refractory CLL and relapsed B-cell ALL both demonstrated progress following second-generation CAR T cell therapy (61, 62). A retroviral vector named MSGV was utilized to deliver a CD19-specific CAR as part of the treatment at NCI. This CAR was designed to target the CD19 protein found on the surface of B-lineage cells, using an anti-CD19 scFv derived from the murine monoclonal antibody FMC63. It incorporated both a CD3z endodomain and a CD28 costimulatory endodomain. Following lymphodepletion, the patient received two infusions of CAR T cells and eight doses of IL-2. As a result of this therapy, the patient underwent selective elimination of B-lineage cells and achieved a partial remission of the lymphoma (61). Autologous CD19-targeted CAR T cells featuring the second-generation CAR (19-28z) were evaluated for safety and long-lasting effects in patients with B-ALL and CLL that had either relapsed or were resistant to chemotherapy in the MSKCC Phase 1 trial. Patients who had previously received cyclophosphamide training exhibited a partial response, whereas those who had not been trained showed no measurable reactions to their disease (62).

When Dr. Carl June and his team at the University of Pennsylvania shared their findings that three adult patients suffering from advanced chronic lymphocytic leukemia (CLL) achieved either complete or partial remission after undergoing CD19-specific CAR T cell therapy, it represented a major breakthrough in the application of CAR T cell therapy(63,64).

The construct utilized in this trial included the 4-1BB costimulatory endodomain, the CD3z signaling endodomain, and an anti-CD19 scFv derived from FMC63. An EF1-a promoter-driven lentiviral vector was employed to express this construct. Following injection, the CAR T cell counts in patients increased significantly, often by a factor of 1,000. These results enabled the treatment of advanced cases of CLL and other B-cell malignancies using second-generation CAR T cell therapy.

The outcomes of these clinical studies revealed that lymphodepletion prior to treatment—specifically a type of chemotherapy that reduces the immune cell count—is essential for the success of CAR T cell therapy. Conversely, it appears that IL-2 is not necessary. Dr. Steven Rosenberg's team was the first to demonstrate that lymphodepleting chemotherapy is effective. They found that the combination of cyclophosphamide and fludarabine led to the in vivo growth and movement of injected tumor-reactive T cells toward tumor locations (65–67). The process of lymphodepletion might include reducing the count of native lymphocytes that compete with the infused T cells and increasing the circulating levels of T cell growth factors such as IL-15. This would promote more effective growth of the administered T cells within the host's body (68).

### 1.16. Challenges for CAR-T cell therapy

Although CAR-T cells have been utilized in clinical settings, several questions persist, including the most suitable vector and the safety profile over the long term. A crucial element of the CAR-T cell product is the viral vector responsible for introducing the CARs into the T cells. Large quantities of the CAR-encoding viral vector can be produced and stored at -80 °C for as long as nine years (69). Since these cells will be infused into the patients, it is crucial for the vector to be sterile. The third generation appears to be the safest minimum lentiviral vector found so far (70). It is essential to

conduct quality control testing on safety, sterility, titer, purity, and potency. Given that there are multiple sources for vectors, it is vital to evaluate their function, stability, and purity. Ideally, CAR-T cells should be produced using a single vector while monitoring these variables. A concern that arises is the potential for insertional mutagenesis resulting from the integration of vector DNA into host cells. In comparison to other vectors, lentiviral vectors might pose a lower risk of mutagenesis (71). On another note, retroviral and lentiviral vectors possess the potential to induce cancer. The long-term safety of using viral vectors in CAR-T cell therapy remains uncertain. Thus, it is vital to monitor any possible long-term negative effects linked to these vectors. The impact of durable CAR-T cells on future pregnancies is still not fully understood.

To ensure effective handling of materials and patient scheduling throughout the therapy, there needs to be seamless coordination among the collection, manufacturing, and treatment locations. Consequently, establishing a standardized manufacturing process for CAR-T cells is essential to identify the critical quality attributes and desired product characteristics. Furthermore, since various products exhibit differing CAR viability, phenotype, and positivity, gaining deeper insights into these processes is important (72). Leukapheresis produces a diverse array of starting materials, making it difficult to compare the resulting products.

While CAR-T cell therapy has demonstrated encouraging results in treating hematological cancers, solid tumors present ongoing challenges due to immune-suppressive tumor environments, the loss of antigens in tumor cells, and a shortage of specific antigens (73, 74). Consequently, notable progress has been achieved by integrating CARs with various effector molecules; including PD1 switch receptors, anti-oxidant enzymes, matrix degradation enzymes, and others, to enhance the efficacy of CAR-T cell therapy (75–79). Severe cytokine release syndrome is the primary side effect associated with CAR-T cell therapy (CRS). It is vital to minimize side effects without compromising therapeutic effectiveness, even though most adverse reactions following CAR-T cell therapy can be managed with existing treatments. Similarly, improving the safety of CAR-T cell therapy is essential for increasing the specificity of the modified T cells (80).

## 2. The major challenges include

### 2.1. Severe adverse events

CAR T cell therapy has shown great promise in the treatment of hematological cancers, but one major concern with this approach is the potential for life-threatening adverse events. Two of the most common adverse events are cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS) (81).CRS is mediated by the cytokines IL-1 and IL-6, which can cause fever, hypotension, and other systemic symptoms. To mitigate the risk of CRS and neurotoxicity, the FDA approved the use of the humanized anti-IL6 receptor antibody tocilizumab in 2017 for CAR T cell therapy. Other potential treatments for these adverse events include the IL-1 receptor antagonist anakinra and the anti-IL6 chimeric antibody siltuximab(82). The severity of adverse events associated with CAR T cell therapy is influenced by several factors, including the pretreatment tumor burden, lymphodepletion regimen intensity, and CAR T cell dose (83). During CD19-CAR T cell therapy, elevated cytokine levels have been associated with prior tumor burden (84, 85). Tumor load can impede the rates of full remission and potentially overall survival, but it does not seem to influence CD19-CAR T cell growth peaks (86).

### 2.2. High cost of manufacturing autologous CAR T cells

One drawback of the present CAR T cell therapy is the expensive charges of producing autologous CAR T cells, which can cause patients with severe CRS to spend up to $500,000 on treatment overall (87). It also takes between 21 and 35 days on average to manufacture autologous CAR T cells. During this waiting period, patients might need bridging therapy and occasionally pass away from quickly progressing illness without receiving the benefits of CAR T cell therapy. Additionally, T cells from healthy donors may be more active than T cells from sick patients due to tiredness. Therefore, a number of approaches are being investigated to make this treatment more accessible and economical, such as the use of commercially available allogenic CAR (allo-CAR) T cells and *in vivo* CAR T cell production. Generating CAR T cells in vivo is an intriguing cost-effective tactic. By delivering mRNA encoding the FAP-targeting CAR in lipid nanoparticles (LNPs), transient CAR T cells can be produced in vivo (88).

## 3. Conclusion

CAR-T treatment for patients with tumors has shown promising outcomes; however, many remaining challenges need to be considered. The high quality of CAR-T products needs to be ensured through optimization of protocols, and the long-term safety requires further study.

## Compliance with ethical standards

*Disclosure of conflict of interest*

No conflict of interest to be disclsoed.

## References


[1] Parikh RH, Lonial S. Chimeric antigen receptor T-cell therapy in multiple myeloma: A comprehensive review of current data and implications for clinical practice. CA Cancer J Clin. 2023 May 10; 73(3):275–85.

[2] Mohanty R, Chowdhury C, Arega S, Sen P, Ganguly P, Ganguly N. CAR T cell therapy: A new era for cancer treatment (Review). Oncol Rep. 2019 Sep 24;

[3] Sterner RC, Sterner RM. CAR-T cell therapy: current limitations and potential strategies. Vol. 11, Blood Cancer Journal. Springer Nature; 2021.

[4] Turtle CJ, Hanafi LA, Berger C, Gooley TA, Cherian S, Hudecek M, et al. CD19 CAR–T cells of defined CD4+:CD8+ composition in adult B cell ALL patients. Journal of Clinical Investigation. 2016 Apr 25; 126(6):2123–38.

[5] Wang Z, Wu Z, Liu Y, Han W. New development in CAR-T cell therapy. Vol. 10, Journal of Hematology and Oncology. BioMed Central Ltd.; 2017.

[6] Zhang C, Liu J, Zhong JF, Zhang X. Engineering CAR-T cells. Biomark Res. 2017 Dec 24; 5(1):22.

[7] Ramos CA, Dotti G. Chimeric antigen receptor (CAR)-engineered lymphocytes for cancer therapy. Expert Opin Biol Ther. 2011 Jul 4; 11(7):855–73.

[8] Lipowska-Bhalla G, Gilham DE, Hawkins RE, Rothwell DG. Targeted immunotherapy of cancer with CAR T cells: achievements and challenges. Cancer Immunology, Immunotherapy. 2012 Jul 22; 61(7):953–62.

[9] Mitra A, Barua A, Huang L, Ganguly S, Feng Q, He B. From bench to bedside: the history and progress of CAR T cell therapy. Front Immunol. 2023 May 15; 14.

[10] S. Bridgeman J, E. Hawkins R, A. Hombach A, Abken H, E. Gilham D. Building Better Chimeric Antigen Receptors for Adoptive T Cell Therapy. Curr Gene Ther. 2010 Apr 1; 10(2):77–90.

[11] Kalos M. Muscle CARs and TcRs: turbo-charged technologies for the (T cell) masses. Cancer Immunology, Immunotherapy. 2012 Jan 1; 61(1):127–35.

[12] Liu J, Zhong JF, Zhang X, Zhang C. Allogeneic CD19-CAR-T cell infusion after allogeneic hematopoietic stem cell transplantation in B cell malignancies. J Hematol Oncol. 2017 Dec 31; 10(1):35.

[13] Dobosz P, Dzieciątkowski T. The Intriguing History of Cancer Immunotherapy. Front Immunol. 2019 Dec 17; 10.

[14] Pearl R. On the Pathological Relations between Cancer and Tuberculosis. Exp Biol Med. 1928 Oct 1; 26(1):73–5.

[15] Morales A, Eidinger D, Bruce AW. Intracavitary Bacillus Calmette-guerin in the Treatment of Superficial Bladder Tumors. Journal of Urology. 1976 Aug; 116(2):180–2.

[16] Kuwana Y, Asakura Y, Utsunomiya N, Nakanishi M, Arata Y, Itoh S, et al. Expression of chimeric receptor composed of immunoglobulin-derived V resions and T-cell receptor-derived C regions. Biochem Biophys Res Commun. 1987 Dec; 149(3):960–8.

[17] Gross G, Waks T, Eshhar Z. Expression of immunoglobulin-T-cell receptor chimeric molecules as functional receptors with antibody-type specificity. Proceedings of the National Academy of Sciences. 1989 Dec; 86(24):10024–8.

[18] Eshhar Z, Waks T, Gross G, Schindler DG. Specific activation and targeting of cytotoxic lymphocytes through chimeric single chains consisting of antibody-binding domains and the gamma or zeta subunits of the immunoglobulin and T-cell receptors. Proceedings of the National Academy of Sciences. 1993 Jan 15; 90(2):720–4.

[19] Brocker T. Chimeric Fv-ζ or Fv-ε receptors are not sufficient to induce activation or cytokine production in peripheral T cells. Blood. 2000 Sep 1; 96(5):1999–2001.

[20] Heuser C, Hombach A, Lösch C, Manista K, Abken H. T-cell activation by recombinant immunoreceptors: Impact of the intracellular signalling domain on the stability of receptor expression and antigen-specific activation of grafted T cells. Gene Ther. 2003 Aug 1; 10(17):1408–19.

[21] Zhao Y, Wang QJ, Yang S, Kochenderfer JN, Zheng Z, Zhong X, et al. A Herceptin-Based Chimeric Antigen Receptor with Modified Signaling Domains Leads to Enhanced Survival of Transduced T Lymphocytes and Antitumor Activity. The Journal of Immunology. 2009 Nov 1; 183(9):5563–74.

[22] Bridgeman JS, Hawkins RE, Bagley S, Blaylock M, Holland M, Gilham DE. The Optimal Antigen Response of Chimeric Antigen Receptors Harboring the CD3ζ Transmembrane Domain Is Dependent upon Incorporation of the Receptor into the Endogenous TCR/CD3 Complex. The Journal of Immunology. 2010 Jun 15; 184(12):6938–49.

[23] Kershaw MH, Westwood JA, Parker LL, Wang G, Eshhar Z, Mavroukakis SA, et al. A Phase I Study on Adoptive Immunotherapy Using Gene-Modified T Cells for Ovarian Cancer. Clinical Cancer Research. 2006 Oct 15; 12(20):6106–15.

[24] Park JR, Digiusto DL, Slovak M, Wright C, Naranjo A, Wagner J, et al. Adoptive Transfer of Chimeric Antigen Receptor Re-directed Cytolytic T Lymphocyte Clones in Patients with Neuroblastoma. Molecular Therapy [Internet]. 2007; 15(4):825–33.

[25] Lamers CHJ, Langeveld SCL, Groot-van Ruijven CM, Debets R, Sleijfer S, Gratama JW. Gene-modified T cells for adoptive immunotherapy of renal cell cancer maintain transgene-specific immune functions in vivo. Cancer Immunology, Immunotherapy. 2007 Dec 4; 56(12):1875–83.

[26] Pule MA, Savoldo B, Myers GD, Rossig C, Russell H V, Dotti G, et al. Virus-specific T cells engineered to coexpress tumor-specific receptors: persistence and antitumor activity in individuals with neuroblastoma. Nat Med. 2008 Nov 2; 14(11):1264–70.

[27] Till BG, Jensen MC, Wang J, Chen EY, Wood BL, Greisman HA, et al. Adoptive immunotherapy for indolent non-Hodgkin lymphoma and mantle cell lymphoma using genetically modified autologous CD20-specific T cells. Blood. 2008 Sep 15; 112(6):2261–71.

[28] Finney HM, Lawson ADG, Bebbington CR, Weir ANC. Chimeric Receptors Providing Both Primary and Costimulatory Signaling in T Cells from a Single Gene Product. The Journal of Immunology. 1998 Sep 15; 161(6):2791–7.

[29] Dotti G, Savoldo B, Brenner M. Fifteen Years of Gene Therapy Based on Chimeric Antigen Receptors: "Are We Nearly There Yet?" Hum Gene Ther. 2009 Nov; 20(11):1229–39.

[30] Park TS, Rosenberg SA, Morgan RA. Treating cancer with genetically engineered T cells. Trends Biotechnol. 2011 Nov; 29(11):550–7.

[31] Acuto O, Michel F. CD28-mediated co-stimulation: a quantitative support for TCR signalling. Nat Rev Immunol. 2003 Dec; 3(12):939–51.

[32] Finney HM, Akbar AN, Lawson ADG. Activation of Resting Human Primary T Cells with Chimeric Receptors: Costimulation from CD28, Inducible Costimulator, CD134, and CD137 in Series with Signals from the TCRζ Chain. The Journal of Immunology. 2004 Jan 1; 172(1):104–13.

[33] Croft M. The role of TNF superfamily members in T-cell function and diseases. Nat Rev Immunol. 2009 Apr; 9(4):271–85.

[34] Cai B, Guo M, Wang Y, Zhang Y, Yang J, Guo Y, et al. Co-infusion of haplo-identical CD19-chimeric antigen receptor T cells and stem cells achieved full donor engraftment in refractory acute lymphoblastic leukemia. J Hematol Oncol. 2016 Dec 25;9(1):131.

[35] Hu Y, Sun J, Wu Z, Yu J, Cui Q, Pu C, et al. Predominant cerebral cytokine release syndrome in CD19-directed chimeric antigen receptor-modified T cell therapy. J Hematol Oncol. 2016 Dec 15; 9(1):70.

[36] Porter DL, Hwang WT, Frey N V., Lacey SF, Shaw PA, Loren AW, et al. Chimeric antigen receptor T cells persist and induce sustained remissions in relapsed refractory chronic lymphocytic leukemia. Sci Transl Med. 2015 Sep 2; 7(303).

[37] Frigault MJ, Lee J, Basil MC, Carpenito C, Motohashi S, Scholler J, et al. Identification of Chimeric Antigen Receptors That Mediate Constitutive or Inducible Proliferation of T Cells. Cancer Immunol Res. 2015 Apr 1; 3(4):356–67.

[38] Long AH, Haso WM, Shern JF, Wanhainen KM, Murgai M, Ingaramo M, et al. 4-1BB costimulation ameliorates T cell exhaustion induced by tonic signaling of chimeric antigen receptors. Nat Med. 2015 Jun 4; 21(6):581–90.

[39] Kawalekar OU, O'Connor RS, Fraietta JA, Guo L, McGettigan SE, Posey AD, et al. Distinct Signaling of Coreceptors Regulates Specific Metabolism Pathways and Impacts Memory Development in CAR T Cells. Immunity. 2016 Feb; 44(2):380–90.

[40] Marin V, Pizzitola I, Agostoni V, Attianese GMPG, Finney H, Lawson A, et al. Cytokine-induced killer cells for cell therapy of acute myeloid leukemia: improvement of their immune activity by expression of CD33-specific chimeric receptors. Haematologica. 2010 Dec 1; 95(12):2144–52.

[41] Till BG, Jensen MC, Wang J, Qian X, Gopal AK, Maloney DG, et al. CD20-specific adoptive immunotherapy for lymphoma using a chimeric antigen receptor with both CD28 and 4-1BB domains: pilot clinical trial results. Blood. 2012 Apr 26; 119(17):3940–50.

[42] Morgan RA, Yang JC, Kitano M, Dudley ME, Laurencot CM, Rosenberg SA. Case Report of a Serious Adverse Event Following the Administration of T Cells Transduced With a Chimeric Antigen Receptor Recognizing ERBB2. Molecular Therapy. 2010 Apr; 18(4):843–51.

[43] Chmielewski M, Abken H. TRUCKs: the fourth generation of CARs. Expert Opin Biol Ther. 2015 Aug 3; 15(8):1145–54.

[44] Sha H huan, Wang D dan, Yan D li, Hu Y, Yang S jin, Liu S wen, et al. Chimaeric antigen receptor T-cell therapy for tumour immunotherapy. Biosci Rep. 2017 Feb 28; 37(1).

[45] Bourbon E, Ghesquières H, Bachy E. CAR-T cells, from principle to clinical applications. Bull Cancer. 2021 Oct; 108(10):S4–17.

[46] Sadelain M, Brentjens R, Rivière I. The Basic Principles of Chimeric Antigen Receptor Design. Cancer Discov. 2013 Apr 1; 3(4):388–98.

[47] Galluzzi L, Martin P. CARs on a highway with roadblocks. Oncoimmunology. 2017 Dec 2; 6(12):e1388486.

[48] Hu WS, Pathak VK. Design of retroviral vectors and helper cells for gene therapy. Pharmacol Rev. 2000 Dec; 52(4):493–511.

[49] Wang GP, Garrigue A, Ciuffi A, Ronen K, Leipzig J, Berry C, et al. DNA bar coding and pyrosequencing to analyze adverse events in therapeutic gene transfer. Nucleic Acids Res. 2008 May; 36(9):e49–e49.

[50] El-Aneed A. An overview of current delivery systems in cancer gene therapy. Journal of Controlled Release. 2004 Jan; 94(1):1–14.

[51] Pahle J, Walther W. Vectors and strategies for nonviral cancer gene therapy. Expert Opin Biol Ther. 2016 Apr 2; 16(4):443–61.

[52] Chen ZY, He CY, Kay MA. Improved Production and Purification of Minicircle DNA Vector Free of Plasmid Bacterial Sequences and Capable of Persistent Transgene Expression *In Vivo*. Hum Gene Ther. 2005 Jan; 16(1):126–31.

[53] Kay MA, He CY, Chen ZY. A robust system for production of minicircle DNA vectors. Nat Biotechnol. 2010 Dec 21; 28(12):1287–9.

[54] Levine BL. Performance-enhancing drugs: design and production of redirected chimeric antigen receptor (CAR) T cells. Cancer Gene Ther. 2015 Feb 13;22(2):79–84.

[55] Lee G, Arepally GM. Anticoagulation techniques in apheresis: From heparin to citrate and beyond. J Clin Apher. 2012 Jan 24; 27(3):117–25.

[56] Guedan S, Chen X, Madar A, Carpenito C, McGettigan SE, Frigault MJ, et al. ICOS-based chimeric antigen receptors program bipolar TH17/TH1 cells. Blood. 2014 Aug 14; 124(7):1070–80.

[57] McGarrity GJ, Hoyah G, Winemiller A, Andre K, Stein D, Blick G, et al. Patient monitoring and follow-up in lentiviral clinical trials. J Gene Med. 2013 Feb 27; 15(2):78–82.

[58] Beatty GL, Haas AR, Maus M V., Torigian DA, Soulen MC, Plesa G, et al. Mesothelin-Specific Chimeric Antigen Receptor mRNA-Engineered T Cells Induce Antitumor Activity in Solid Malignancies. Cancer Immunol Res. 2014 Feb 1; 2(2):112–20.

[59] Bajgain P, Mucharla R, Wilson J, Welch D, Anurathapan U, Liang B, et al. Optimizing the production of suspension cells using the G-Rex "M" series. Mol Ther Methods Clin Dev. 2014; 1:14015.

[60] Mock U, Nickolay L, Philip B, Cheung GWK, Zhan H, Johnston ICD, et al. Automated manufacturing of chimeric antigen receptor T cells for adoptive immunotherapy using CliniMACS Prodigy. Cytotherapy. 2016 Aug; 18(8):1002–11.

[61] Kochenderfer JN, Wilson WH, Janik JE, Dudley ME, Stetler-Stevenson M, Feldman SA, et al. Eradication of B-lineage cells and regression of lymphoma in a patient treated with autologous T cells genetically engineered to recognize CD19. Blood. 2010 Nov 18; 116(20):4099–102.

[62] Brentjens RJ, Rivière I, Park JH, Davila ML, Wang X, Stefanski J, et al. Safety and persistence of adoptively transferred autologous CD19-targeted T cells in patients with relapsed or chemotherapy refractory B-cell leukemias. Blood. 2011 Nov 3; 118(18):4817–28.

[63] Kalos M, Levine BL, Porter DL, Katz S, Grupp SA, Bagg A, et al. T Cells with Chimeric Antigen Receptors Have Potent Antitumor Effects and Can Establish Memory in Patients with Advanced Leukemia. Sci Transl Med. 2011 Aug 10; 3(95).

[64] Porter DL, Levine BL, Kalos M, Bagg A, June CH. Chimeric Antigen Receptor–Modified T Cells in Chronic Lymphoid Leukemia. New England Journal of Medicine. 2011 Aug 25; 365(8):725–33.

[65] Rosenberg SA, Spiess P, Lafreniere R. A New Approach to the Adoptive Immunotherapy of Cancer with Tumor-Infiltrating Lymphocytes. Science (1979). 1986 Sep 19; 233(4770):1318–21.

[66] Dudley ME, Wunderlich JR, Robbins PF, Yang JC, Hwu P, Schwartzentruber DJ, et al. Cancer Regression and Autoimmunity in Patients After Clonal Repopulation with Antitumor Lymphocytes. Science (1979). 2002 Oct 25; 298(5594):850–4.

[67] Dudley ME, Wunderlich JR, Yang JC, Sherry RM, Topalian SL, Restifo NP, et al. Adoptive Cell Transfer Therapy Following Non-Myeloablative but Lymphodepleting Chemotherapy for the Treatment of Patients With Refractory Metastatic Melanoma. Journal of Clinical Oncology. 2005 Apr 1; 23(10):2346–57.

[68] Rosenberg SA, Restifo NP. Adoptive cell transfer as personalized immunotherapy for human cancer. Science (1979). 2015 Apr 3; 348(6230):62–8.

[69] Przybylowski M, Hakakha A, Stefanski J, Hodges J, Sadelain M, Rivière I. Production scale-up and validation of packaging cell clearance of clinical-grade retroviral vector stocks produced in Cell Factories. Gene Ther. 2006 Jan 1; 13(1):95–100.

[70] Dull T, Zufferey R, Kelly M, Mandel RJ, Nguyen M, Trono D, et al. A Third-Generation Lentivirus Vector with a Conditional Packaging System. J Virol. 1998 Nov 1; 72(11):8463–71.

[71] Vannucci L, Lai M, Chiuppesi F, Ceccherini-Nelli L, Pistello M. Viral vectors: a look back and ahead on gene transfer technology. New Microbiol. 2013 Jan; 36(1):1–22.

[72] Porter DL, Levine BL, Kalos M, Bagg A, June CH. Chimeric Antigen Receptor–Modified T Cells in Chronic Lymphoid Leukemia. New England Journal of Medicine. 2011 Aug 25; 365(8):725–33.

[73] Yu S, Li A, Liu Q, Li T, Yuan X, Han X, et al. Chimeric antigen receptor T cells: a novel therapy for solid tumors. J Hematol Oncol. 2017 Dec 29; 10(1):78.

[74] Kato D, Yaguchi T, Iwata T, Morii K, Nakagawa T, Nishimura R, Prospects for personalized combination immunotherapy for solid tumors based on adoptive cell therapies and immune checkpoint blockade therapies. Japanese Journal of Clinical Immunology. 2017; 40(1):68–77.

[75] Morsut L, Roybal KT, Xiong X, Gordley RM, Coyle SM, Thomson M, et al. Engineering Customized Cell Sensing and Response Behaviors Using Synthetic Notch Receptors. Cell. 2016 Feb; 164(4):780–91.

[76] Roybal KT, Rupp LJ, Morsut L, Walker WJ, McNally KA, Park JS, et al. Precision Tumor Recognition by T Cells With Combinatorial Antigen-Sensing Circuits. Cell. 2016 Feb; 164(4):770–9.

[77] Newick K, O'Brien S, Sun J, Kapoor V, Maceyko S, Lo A, et al. Augmentation of CAR T-cell Trafficking and Antitumor Efficacy by Blocking Protein Kinase A Localization. Cancer Immunol Res. 2016 Jun 1; 4(6):541–51.

[78] Caruana I, Savoldo B, Hoyos V, Weber G, Liu H, Kim ES, et al. Heparanase promotes tumor infiltration and antitumor activity of CAR-redirected T lymphocytes. Nat Med. 2015 May 13; 21(5):524–9.

[79] Rodgers DT, Mazagova M, Hampton EN, Cao Y, Ramadoss NS, Hardy IR, et al. Switch-mediated activation and retargeting of CAR-T cells for B-cell malignancies. Proceedings of the National Academy of Sciences. 2016 Jan 26;113(4).

[80] Lee DW, Gardner R, Porter DL, Louis CU, Ahmed N, Jensen M, et al. Current concepts in the diagnosis and management of cytokine release syndrome. Blood. 2014 Jul 10; 124(2):188–95.

[81] Ferreros P, Trapero I. Interleukin inhibitors in cytokine release syndrome and neurotoxicity secondary to CAR-T therapy. *Diseases* (2022) 10(3):41. doi: 10.3390/diseases10030041.

[82] Narkhede M, Di Stasi A, Bal S, Shea LK, Goyal G, Sledge A, Interim analysis of investigator-initiated phase 2 trial of siltuximab in treatment of cytokine release syndrome and immune effector cell associated neurotoxicity related to CAR T-cell therapy. In: *2023* tandem meetings| transplantation & cellular therapy meetings of ASTCT and CIBMTR. Tandem Meetings (2023).

[83] Hay KA, Hanafi LA, Li D, Gust J, Liles WC, Wurfel MM, et al. Kinetics and biomarkers of severe cytokine release syndrome after CD19 chimeric antigen receptor-modified T-cell therapy. *Blood* (2017) 130:2295–306. doi: 10.1182/blood-2017-06-793141

[84] Brentjens RJ, Davila ML, Riviere I, Park J, Wang X, Cowell LG, et al. CD19- targeted T cells rapidly induce molecular remissions in adults with chemotherapyrefractory acute lymphoblastic leukemia. Sci Transl Med (2013) 5:177ra138. doi: 10.1126/scitranslmed.3005930

[85] Davila ML, Riviere I, Wang X, Bartido S, Park J, Curran K, et al. Efficacy and toxicity management of 19-28z CAR T cell therapy in b cell acute lymphoblast

[86] Li M, Xue SL, Tang X, Xu J, Chen S, Han Y, et al. The differential effects of tumor burdens on predicting the net benefits of ssCART-19 cell treatment on r/r b-ALL patients. Sci Rep (2022) 12:378. doi: 10.1038/s41598-021-04296-3

[87] Hernandez I, Prasad V, Gellad WF. Total costs of chimeric antigen receptor Tcell immunotherapy. JAMA Oncol (2018) 4:994–6. doi: 10.1001/jamaoncol.2018.0977

[88] Rurik JG, Tombacz I, Yadegari A, Mendez Fernandez PO, Shewale SV, Li L, et al. CAR T cells produced in vivo to treat cardiac injury. Science (2022) 375:91–6. doi: 10.1126/science.abm0594